Summary of Invention

The invention is drawn to methods of producing purified caveolae, the methods including an immunoisolation step of incubating a sample containing plasma membranes with an antibody that is specific for caveolin and which binds to oligomerized caveolin in its native state as an oligomeric structural cage surrounding intact caveolae. The methods are simple and efficient means of producing purified caveolae which closely resemble caveolae in their native state (e.g., caveolae covered with the oligomeric structural cage of caveolin); the methods also minimize contamination and loss of molecules that dissociate from caveolae over time. Furthermore, the methods do not require perfusion of a tissue or coating of the plasma membranes with colloidal silica (described, for example, in US Patent 5,776,770), and thus allow a high level of flexibility of starting materials, as the methods can be used even for tissues or samples that cannot be perfused or coated with colloidal silica.

Rejection of Claims under 35 U.S.C. 112, first paragraph

The Examiner rejected Claims 1-9, 11-17, 19-22 and 24-25, stating that the specification did not provide enablement for a monoclonal antibody which binds oligomerized caveolin in its native state as an oligomeric structural cage surrounding intact caveolae other than CAV (mAb clone 2234).

Applicant respectfully disagree with this assessment. Applicants' disclosure describes the use of one antibody, CAV, which is representative of a type of antibody with a specific characteristic, namely, the ability to bind oligomerized caveolin in its native state as an oligomeric structural cage surrounding intact caveolae. The inability of other antibodies used in the experiments described in the disclosure, or described in Oh and Schnitzer, to bind to oligomerized caveolin in its native state as an oligomeric structural cage surrounding intact caveolae, indicates only that those antibodies lack this particular characteristic and that a different antibody having this characteristic should be used in the methods of the invention. The Specification describes experiments by which the ability of an antibody to bind to caveolin in its native state as an oligomeric structural cage surrounding intact caveolae can be determined (see, e.g., p. 13, line 11 *et seq.*). One of ordinary skill in the art, given the screening criteria and the description of the specific characteristic of the antibody (i.e., the ability to bind oligomerized caveolin in its native state as an oligomeric structural cage surrounding intact caveolae) in the Specification, as well as the methods of determining the binding characteristics of the antibody in

the Specification, would be able to identify other antibodies having the desired characteristics without undue experimentation.

Rejection of Claims under 35 U.S.C. §102(b)

Claims 1-4, 6, 11 and 14 have been rejected under 35 U.S.C. §102(b), because the Examiner contends that they are anticipated by Scherer *et al.* The Examiner states that Scherer *et al.* teach two monoclonal antibodies, including mAb 2234 (i.e., CAV antibody), which are "used in immunoprecipitation assays to purify caveolae."

In order for a reference to anticipate claims, the reference must teach every aspect of the claimed invention either explicitly or impliedly (see M.P.E.P. § 2131).

Scherer *et al.* describe a method of immunoprecipitation of caveolin. They indicate that three different caveolin genes (Cav-1, Cav-2, and Cav-3) encoding four different subtypes of caveolin have been described, and that study of caveolin-2 has been hampered by a lack of caveolin-2-specific antibodies. They describe a mAb that recognizes caveolin-2 protein but not other known members of the caveolin gene family, and characterize expression and localization of caveolin-2 protein using that antibody. They utilize CAV (mAb 2234), which binds to caveolin-1 for immunoprecipitation.

Immunoprecipitation of caveolin, as described by Scherer *et al.*, differs significantly from immunoisolation of caveolae as described in the Specification. Immunoprecipitation refers to separation, usually of a single protein (in this case, caveolin), from the environment in which it is found (here, from a cell lysate) using an antibody. In the immunoprecipitation described by Scherer *et al.*, the cells are lysed in the presence of detergent (see "*Immunoprecipitation*" discussion), which not only disrupts but also destroys membranes, and strips lipids as well as proteins from cellular components, thereby exposing caveolin and allowing the antibody to bind to it. Thus, the methods of Scherer *et al.* strip away both lipids and other proteins attached to caveolin, disrupting the structure of the caveolae and thereby eliminating the possibility of isolating the caveolae themselves. Scherer *et al.* do not describe subjection of a sample comprising any plasma membranes to any immunoisolation method.

In contrast to the methods described by Scherer *et al.*, the immunoisolation described in the Specification separates a whole, complex organelle (a caveola) from plasma membranes of a cell, using an antibody. In the immunoisolation methods of the invention, a sample comprising plasma membranes is used; these membranes must be present in order to perform the methods of

isolating caveolae, as the caveolae are organelles that are an integral part of the membranes. The caveolae are then separated from the plasma membranes. Scherer *et al.* do not teach isolation of caveolae, but only isolation of caveolin.

In view of these considerations, it is clear that Scherer *et al.* do not describe isolation of caveolae from a sample comprising plasma membranes, as in the claimed methods of the invention. Thus, Scherer *et al.* do not teach every aspect of the claimed invention either explicitly or impliedly, and the claimed invention is therefore not anticipated by the teachings of Scherer *et al.*

CONCLUSION

In view of the discussion presented above, the claims are in condition for allowance. Applicants respectfully request that the Examiner reconsider and withdraw all rejections.

If the Examiner believes that a telephone conversation would expedite prosecution, the Examiner is invited to contact Elizabeth W. Mata at (915) 845-3558. If Elizabeth W. Mata cannot be reached, the Examiner is invited to contact Doreen Hogle at (978) 341-0036

Respectfully submitted,

HAMILTON, BROOK, SMITH & REYNOLDS, P.C.

By Doreen M. Hogle Reg. No. 36,361 for
Elizabeth W. Mata
Registration No. 38,236
Telephone (978) 341-0036
Facsimile (978) 341-0136

Concord, MA 01742-9133
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